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Stability of oral liquid preparations of methylergometrine

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Received August 3, 2005, accepted October 22, 2005

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Pharmazie 61: 701–705 (2006)

The stability of an oral ready to-use form of methylergometrine (0.05 mg/mL), which provides a convenient volume for administration (5 mL), was evaluated over a forty-seven-day period at different temperatures (5 °C and room temperature) without light in order to assign a shelf life. Methylergometrine was assayed by a stability-indicating HPLC method with diode array detection. The drug undergoes degradation under basic conditions and dry heat (50 °C). All the peaks of the degraded product were resolved from the standard drug with significantly different retention times. Statistical analysis proves that the method is reproducible and accurate for estimation of the intact drug. The pH of samples was monitored periodically for changes. Samples were also visually inspected for any colour change, precipitation or crystallization. At least, 96% of the initial methylergometrine concentration remained throughout the 47-day study period. Over the test period, no significant change was observed in the pH or colour of any of the samples. No degradation products were revealed. This study allowed an oral ready to use solution of methylergometrine (0.05 mg/ml) to be prepared, with a shelf life of more than one month (47 days) when stored at room temperature without light.

1. Introduction

Known for its strong uterotonic effect resulting in reduced loss of blood, methylergometrine or methylergonovine maleate (9,10-didehydro-*N*-[1-(hydroxymethyl)propyl]-6-methylergoline-8-carboxamide) is widely used in obstetrics and gynaecology (Vishnevskii et al. 1970), and is classified as an essential drug in the WHO Model List of Essential Drugs (Hogerzeil et al. 1996) for the prevention and treatment of *post partum* and *post abortion* haemorrhages (De Groot et al. 1998; Martindale 2004).

In France, methylergometrine maleate (Methergin®) is available in oral tablets (0.125 mg), oral solution (0.25 mg/mL) and injectable solution (0.2 mg/mL). The recommended dose for oral Methergin® is usually 0.25 mg (1 mL) three times daily.

In order to improve the safety of the distribution system, limit waste of drugs and be convenient for nurses and caregivers, a centralized unit-dose dispensing system has been organized in our Department of Pharmacy. We thus prepared an oral unit-dose solution of methylergometrine, with a convenient volume for administration of 5 mL. This was achieved by diluting the commercially available oral solution, Methergin®, in a glycero-alcoholic excipient.

Owing to the possible dilution effect, we evaluated the stability of this preparation, particularly as attention has been drawn to some problems of stability of methylergometrine, and there are considerable differences of stability between different brand-name formulations of the drug. It is recommended by the manufacturer of Methergin® that the oral and injectable solutions should be stored protected from light. Moreover, injectable methylergometrine maleate proved to be very unstable when stored unrefrigerated, the deterioration being more significant when stored at a high temperature, particularly in a tropical climate (Hogerzeil et al. 1992; De Groot et al. 1995) and exposed to light (Hogerzeil et al. 1996; De Groot et al. 1998). The oral tablets also showed deterioration, increasing with temperature and humidity (De Groot et al. 1998; Hogerzeil et al. 1992). Likewise, the level of the active ingredient in oral tablets of ergometrine and methylergometrine declines under all conditions (ergometrine and methylergometrine remained stable for 3 and 21 weeks respectively in the least extreme conditions: 6–10 °C and 83–85% humidity) (De Groot et al. 1998).

Thus, the aim of this study was to evaluate the behaviour of methylergometrine maleate over a forty-seven-day period at different temperatures after diluting to a unit-dose. A number of chromatographic methods have been published for the determination of methylergometrine in pharmaceutical preparations or in plasma (Edlund et al. 1981; U.S. Pharmacopeia USP 28-NF 23. 2000; Tokunaga et al. 1983). We chose a HPLC method adapted from the assay described in the USP Pharmacopeia (U.S. Pharmacopeia USP 28-NF 23. 2000).

2. Investigations, results and discussion

2.1. Separation performance

In the accelerated degradation studies, the methylergometrine peak was well resolved from any observed degrada-

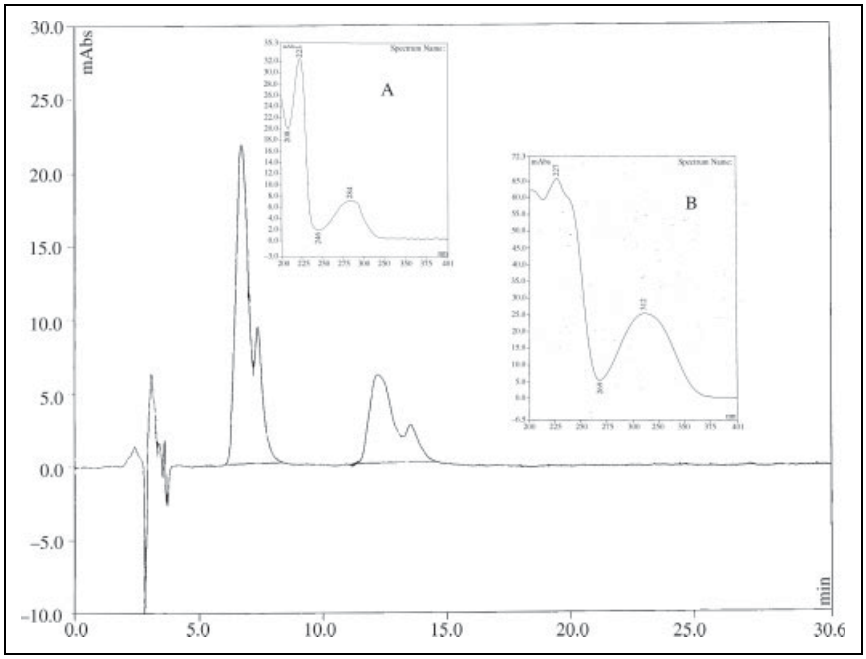


Fig. 1:
HPLC chromatograms of degraded solution and corresponding UV spectra (A = methylergometrine; B = degradation product attributed after analysis at different wavelengths throughout the time of the peak)

tion products (Fig. 1). The purity of the chromatographic peak was confirmed by liquid chromatography equipped with a diode array detector by comparing the UV spectra of the drug peak as it elutes against the standard spectrum of the drug.

Methylergometrine was eluted at about 6.5 min. Major degradation products did not show similar retention times (11.3 min). Thus, degradation products did not interfere with the quantification of methylergometrine.

2.2. Validation of the method

For methylergometrine and unit-dose Methergin®, there was no statistical difference between the slopes of the five-point calibration curves.

For each of the three series, a variance study was done with an AVA program in order to determine if there were statistically significant differences between series. ANOVA gave values of risk <5% in all cases which allows us to conclude that there is no statistically significant difference. The corresponding mean values can therefore be considered representative of the process. The standard curve obtained with pure drug and Methergin gave a relationship between area under the peak and concentration in the range 80–120% which was linear ($r^2 = 0.998$ and $r^2 = 0.997$ respectively for pure drug and Methergin unit-dose) (Table 1).

The within-day coefficients of variation were less than 0.7% (n = 6) and the day-to-day coefficient of variation was less than 2.4% (n = 18) for methylergometrine and Methergin® unit-dose.

The method described in this study allows quantification of methylergometrine.

2.3. Stability study

No precipitation or colour change was observed in the unit-doses of methylergometrine during the 47-day storage period. The observed difference in apparent pH (4.2) for any of the methylergometrine unit dose solutions was less than 5% when stored at 5 or at 21 °C.

The study of the effect of time on stability showed no statistically significant decrease of the concentrations during the 47-day experiment and the chromatogram showed no evidence of chemical degradation products at any time (Fig. 2).

The influence of temperature on the stability of Methergin® unit-doses is shown in Fig. 3. No statistically signifi-

Table 1: Characteristic parameters for the regression equation and comparison of results for determination of pure methylergometrine and Methergin® unit-dose

Parameters	Pure methylergometrine	Methergin® unit-dose	Critical value
Slope			
S.D. of the slope	382.8 ± 21.01	329.8 ± 17.16	
Intercept			
S.D. of the intercept	−1.388 ± 1.06	0.784 ± 0.87	
Coefficient of correlation			
r	0.999	0.998	$r > 0.99$
r^2	0.998	0.997	$r^2 > 0.98$
Homogeneity of variance (Cochran test)	0.6654	0.4448	$<C (0.05; 5; 2) = 0.68$
Significance of the slope	331.94	369.43	$>F (0.01; 1; 13) = 9.07$ $>F (0.001; 1; 13) = 17.81$
F-test	0.1267	0.314	$<F (0.05; 3; 10) = 3.71$
Significance of the intercept	1.308	0.904	$<t (0.05, 13) = 2.16$
t-test to compare intercept	1.58		$<t (0.05, 26) = 2.056$
t-test to compare slope	1.95		$<t (0.05, 26) = 2.056$

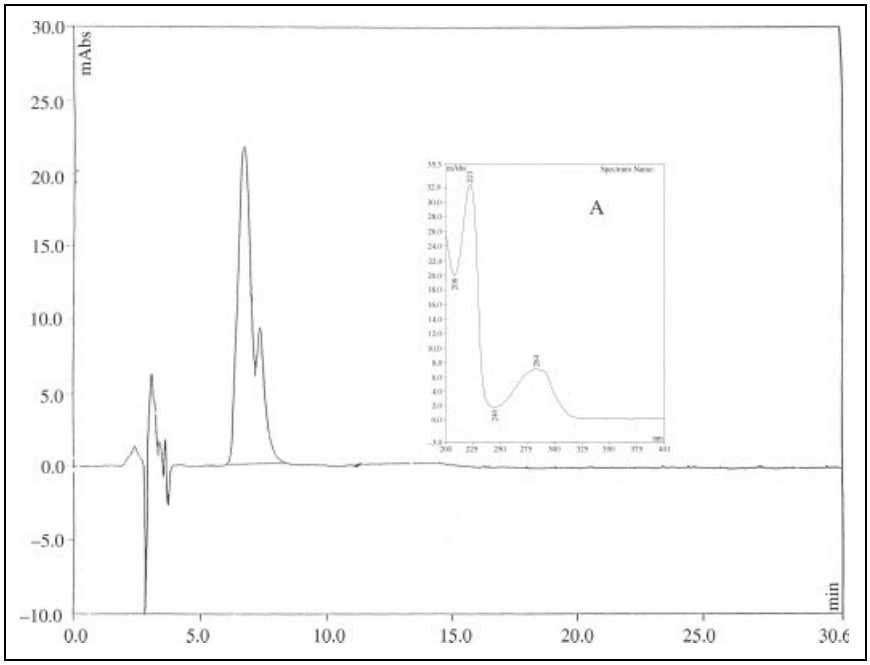


Fig. 2:
HPLC chromatogram of diluted oral unit-dose of methylergometrine maleate obtained at day 0 and day 47 and the corresponding UV spectrum (A) for the same chromatogram

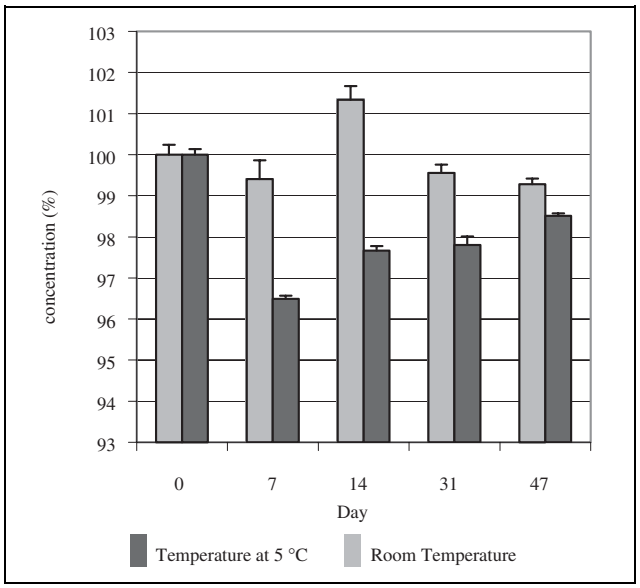


Fig. 3: Evolution of Methergin unit-dose concentration (Day 0 = 100%) at room temperature and 5 °C

cant difference was observed with the Student-t test between group I stored at room temperature, 21 °C and group II at 5 °C.

This result allows more convenient storage at room temperature in obstetrics and gynaecological departments.

At room temperature and 5 °C, the mean concentration of the diluted oral unit-dose of methyl ergometrine maleate was greater than 96% of the initial concentration throughout the 47-day study period. These results confirm those obtained with our previous study by UV-visible spectrophotometry and thin layer chromatography methods (Marigny et al. 2001). Moreover, no degradation products appeared after 47 days (Fig. 2).

Our study provides new information on the long term stability after dilution of methylergometrine maleate. The availability of these stability data allows batch preparation and storage of the diluted oral liquid formulation for 47 days.

Thus, on the basis of our results we decided to prepare ready-to-use unit-doses with a shelf life of 47 days and to store them at room temperature. Microbiological testing was not performed because the unit-dose contains alcohol.

3. Experimental

3.1. Sample preparation

Methylergometrine maleate (Methergin®) (0.25 mg/mL) was purchased from Novartis, Pharma SA (Paris, France) and methylergometrine maleate salt from Sigma-Aldrich (Saint-Quentin, France).

All other reagents were of analytical grade HPLC grade: absolute ethyl alcohol, glycerine Gifrer (Cooper-RPR, Melun, France) and sterile water (Baxter, Maurepas, France), acetonitrile and potassium dihydrogen phosphate (Acros Organics France, Noisy le Grand, France)

3.2. HPLC instrumentation

All chromatographic analyses were carried out using a reverse-phase HPLC system (Kontron Instruments, pump 325) with autoinjector and a photodiode-array detector set at 312 nm (DAD Kontron 550). A 5 µm reverse phase decaoctylsilane C_{18} column (Hypersil 3.9 × 250 mm) was used. Integration and analysis of the data were performed on the Kroma 2000 software controlling the HPLC system. The mobile phase used for the HPLC assay consisted of KH_2PO_4 (pH 4.6; 0.015 M) and acetonitrile in the proportion of 80 : 20 (v/v) and was pumped at 1 mL/min. The mobile phases were filtered under vacuum through 0.45 µm nylon filters and degassed with helium. Separations were performed at room temperature. The HPLC method was as described in the USP pharmacopoeia with minor modifications (U.S. Pharmacopeia, USP 28-NF 23, 2000).

A Mettler-Toledo AC100 Precisa 40SM-200A balance (Greifensee, Switzerland) was used to determine the weight of the unit-dose excipients. The polypropylene unit-doses of 5 ml were provided by Stalo-Medico (Sint-Oedenrode, Holland) and were sealed by air compression. A digital pH meter by Tacussel-Radiometer Analytical (Villeurbanne-France) was also used. The pH meter was calibrated before each measurement.

3.3. Preparation of standard and samples for chemical analysis

A stock solution of methylergometrine was prepared by dissolving 20 mg of methylergometrine maleate in 20 mL of ethyl alcohol**.** Appropriate dilutions were made in the ethyl alcohol to final concentrations between 0.04–0.06 mg/mL for the external standard calibration curve.

A unit-dose of 0.05 mg/mL methylergometrine maleate solution was prepared from Methergin® solution according to the formulation given in Tables 2 and 3. The formulation of the unit-dose solution was based on that of the oral Methergin® solution. Thus, a glycero-alcoholic solvent was prepared for the unit-dose solution (concentration of absolute alcohol 0.05 mg/mL being the same as in the commercial drug Methergin®).

This solution was divided in to polypropylene tubes to obtain 180 unit-doses of 5mL. To prepare the blank solution, Methergin® was replaced by

Table 2: Methylergometrine unit-dose formulation (Methergin® unit-dose)

Methergin®	1 mL (0.25 mg)
Absolute alcohol	0.2 g
Glycerol	1.4 g
Distilled water	5 mL QSP

Table 3: Methergin® oral solution formulation

Methylergometrine maleate (0.25 mg/mL)
Maleic acid, alcohol (0.05 mg/mL)
Glycerol, purified water, carbon dioxide

distilled water. After preparation, the 180 unit-doses were stored, protected from light, half at room temperature (group I, about 20–25 °C), and half at 5 °C (group II). On days 1 to 7, 15, 30 and 47, two unit-doses of each group were analyzed, in duplicate for each unit-dose.

3.4. Stability-indicating studies

The HPLC methods were validated as stability indicating if degradation products can be detected in the same chromatogram as the standard drug. To demonstrate this, it was necessary to carry out accelerated decomposition of methylergometrine in order to obtain degradation products. Thus, a 0.1 mg/mL solution of methylergometrine was subjected to degradation in the presence of humidity, increased temperature, light and atmospheric gases (oxygen). Moreover, the behaviour of methylergometrine in aqueous solution is governed by the pH of the solution and overall basic pH. Although the maleate salt shows improved stability over ergonovine base, the salt can also oxidise and darkens in the presence of oxygen (Scheme). The known degradation products are lumiergonovine I, II, lysergic acid, ergonovinine and iso-lysergic acid (Reif 1982).

Forced hydrolysis in acid and base at a temperature of 50 °C for 4 h was also used. Blank vehicles and samples were degraded under the same conditions as the standards.

3.5. Analytical validation of the HPLC method

The linearity response for methylergometrine maleate and Methergin® unit-dose was determined 3 times from a five-point calibration curve with concentrations in the range 0.04–0.06 mg/mL.

For within-day repeatability, dilutions of methylergometrine and Methergin® unit-dose (0.05 mg/mL) were scanned 6 times. These assays were repeated on three different days for day-to-day repeatability. Coefficient of variation and standard deviation were then calculated.

All the series were performed with a control sample to make sure that the analytical method was valid during the analysis of the samples.

Variation coefficients of less than 2% for within-day assay and less than 4% for day-to-day assay are considered to be acceptable.

The statistical evaluations were performed using two methods: t-test and one-way analysis of variance (ANOVA) with AVA V3-1, Qualilab software following ICH procedures. A maximum risk of 5% of the measures outside the acceptance limits was considered statistically significant.

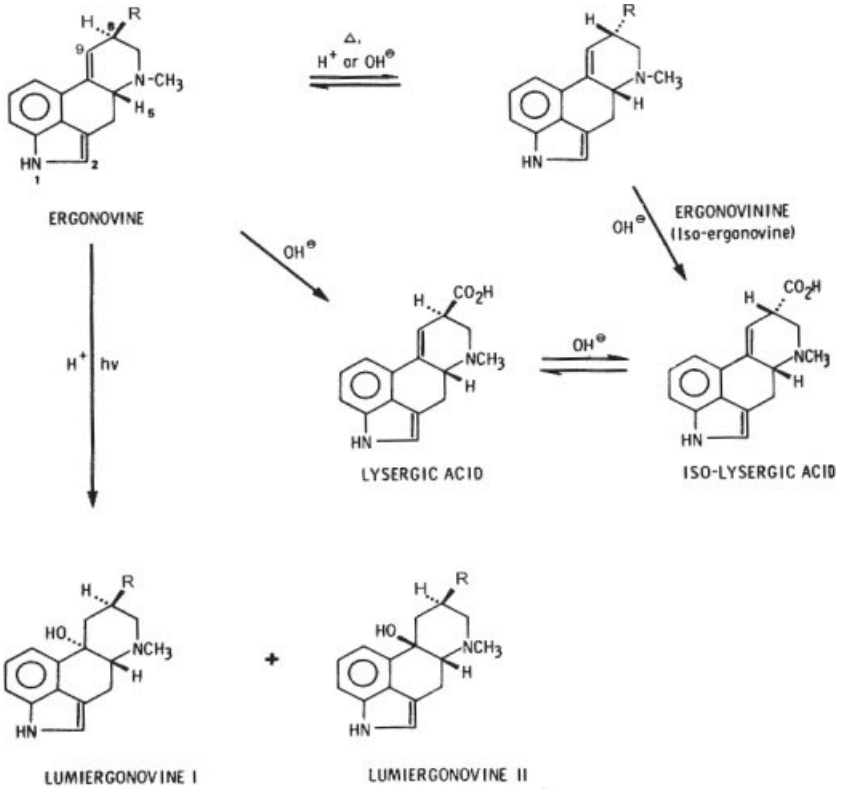
3.6. Physical stability

The physical stability was monitored visually by inspection of the methylergometrine maleate solution on each test day. Variations in pH during the study were considered as a significant indicator for instability. The pH was measured every test day using a Tacussel pH meter calibrated using pH 6 buffers. A preparation is generally considered to be acceptable if the observed difference in pH is less than 5% for any of the solutions.

Though some authors require a shorter shelf life (t_{95} per cent) when the original product is diluted (Mehta 1993), we considered as does Trissel that drug stability was clinically acceptable if 90% or more of the original concentration remained (Trissel et al. 1988; Trissel 2000).

Acknowledgements: We thank the laboratory of pharmacognosy, Faculty of Pharmacy, University of Rennes 1 for the loan of the HPLC-DAD system.

Scheme



R = CONH–CH(CH$_2$OH)–CH$_3$: Ergometrine

R = CONH–CH(CH$_2$OH)–C$_2$H$_5$: Methylergometrine

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